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There is currently little mechanistic data defining the relationship between whole body or spine vibration, physiology and pain. Considering that pain is tremendous problem, a novel model platform for studying how vibration produces chronic pain can provide insight into those exposures with high risk. We hypothesized that a model of vibration and/or jolt induced pain could be produced in the rat. Studies already completed support this hypothesis. Among the major findings is the fact that even 30 minutes of daily vibration for only 7 days is sufficient to induce significant widespread pain that is sustained following the termination of vibration. Another finding is that a host of biochemical changes are associated with pain and some are sensitive to frequency of vibration. Modifications in the cervical disc appear to be the most significant in pilot studies to date. Analysis of transmissibility demonstrates the resonant frequency of the rat spine to be 8Hz, suggesting that the pain response to vibration at that frequency may be even more robust. These findings have tremendous implications for both sub-failure spinal injury and pain. They also establish a strong foundation for the remaining studies including additional exposures and defining the time course of physiological responses.

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## INTRODUCTION

This project combines military injury expertise with in vivo pain modeling to develop in vivo rat models of painful injury mimicking those injuries, in order to serve as a platform system to understand injury risk, biomechanical injury mechanism, and to evaluate measures for injury prevention and treatment. There are three coordinated major activities under this project to ensure we successfully achieve our goals to: (1) identify those activities that are at greatest risk for sustaining and/or inducing injuries to the spine, (2) develop a useful animal model to study these injuries, and (3) establish risk evaluation criteria to identify which injuries and exposures are most threatening. This research project utilizes biomechanical, in vivo and biochemical approaches to define injury and pain mechanisms by which vibration and/or jolt initiates a pain response. We proposed an interdisciplinary research approach between collaborators at an academic research institution and the USAARL, in order to develop effective methods study the most-relevant injuries and to develop a relevant in vivo model system would provide such a tool. In the first year of this project we have made good progress on the development of a painful model of vibration. We have met the associated timeline of activities and milestones that were laid out in the approved statement of work for that effort. We have also performed critical studies to define the anatomic and mechanical scaling differences between the rodent and the human that will be necessary for developing appropriate and meaningful algorithms for evaluating risk for injury as this project moves forward. Lastly, we have initiated studies assessing tissue responses associated with inflammatory, nociceptive and injury responses and uncovered novel relationships between tissue loading, and nerve in-growth, as indicated by NGF, that may be responsible for the pain that develops in our model. With a solid and productive first year of this project we are also poised now to carry out the next set of studies on jolt and the larger animals studies proposed to define the temporal tissue responses that will help shape the remainder of the work on this project.

## BODY

Over the past year of the project, we have made progress on all of the Tasks that were originally proposed to occur during this period of the project. Since the majority of Tasks in the first year required development and pilot testing, we have not yet presented/published work on this project, but we are currently working on 3 abstracts that will be submitted in the next few months, as well as several papers that are developing out of this work. In this portion of the report we include the methods and results for those studies in detail. A primary goal of this work is to develop in vivo rat models of painful injury from vibration and jolt that mimic real-world injuries, in order to serve as a platform system to understand injury risk and biomechanical and biochemical injury mechanisms. To date, the majority of the work has been focused on the anatomic and biomechanical scaling studies, the development of the vibration device and data acquisition system, and initiating the in vivo studies using that system to determine loading profiles. We structure this section of the report to provide an overall summary of each Task and its related status, followed by a more-detailed report of the data and findings for each Task.

The GANTT chart below summarizes the timing of the specific tasks that are associated with each aim across the entire project period under the approved statement of work. Before providing a detailed record of the research findings in this period, we indicate the current status of each activity in that chart to provide an overview of the research activities completed, *ongoing* and *planned*. The majority of activities originally proposed in Year 1 involved obtaining regulatory approvals (Task 1) and initiation of work under Aim 1 and Aim 2 (Tasks 2 & 3). In addition, we also have initiated selected activities under Aims 3 and 4 in the last year, owing to our unexpected progress on Aim 2. Accordingly, we leveraged that progress to advance those efforts and have actually already initiated some studies that were not scheduled to start until Year 2. However, work on Aim 1 has been somewhat delayed due to a delay in obtaining regulatory approval for the human data at USAARL. Nonetheless, we have made progress on that work and expect to be able to achieve those milestones and anticipate completing the

associated tasks in the next year. We provide detailed explanation of these and all Tasks in the following detailed summary broken down for each Task.

| TASK | Year 1 | Year 2 | Year 3 | Year 4 |
|---|---|---|---|---|
| **TASK 1 – Obtain Regulatory Approvals (Y 1)** | | | | |
| 1a. Obtain regulatory approval for animal studies | completed | | | |
| 1b. Obtain regulatory approval for use of human data | *ongoing* | | | |
| **TASK 2 – Aim 1: Review of Injury Exposures in Theater (Ys 1 & 2)** | | | | |
| 2a. Review field data | *ongoing* | | | |
| 2b. Review MARS data | *ongoing* | | | |
| 2c. MARS simulations | *planned* | | | |
| 2d. Revise exposures | *planned* | | | |
| 2e. Publish findings | *planned* | | | |
| **TASK 3 – Aim2: Design Experimental System & Perform Scaled Loading Studies (Ys 1-3)** | | | | |
| 3a. Design initial injury device | completed | | | |
| 3b. Perform scaling studies | *ongoing* | | | |
| 3c. Perform pilot studies with injury device | completed | | | |
| 3d. Modify/redesign device | *ongoing* | | | |
| 3e. Determine loading conditions for in vivo studies | *ongoing* | | | |
| 3f. Perform in vivo studies | *ongoing & planned* | | | |
| 3g. Perform analysis of mechanics | *ongoing & planned* | | | |
| **TASK 4 – Aim 3: Injury Studies for Temporal Characterization (Ys 2-4)** | | | | |
| 4a. Identify injury conditions | | | *ongoing & planned* | |
| 4b. Perform tissue assays for Aim 2 | | *ongoing & planned* | | |
| 4c. Perform injuries | | | *ongoing & planned* | |
| 4d. Perform tissue assays for Aim 3 | | | | *planned* |
| 4e. Integrate findings from Aims 2 and 3 | *ongoing & planned* | | | |
| **TASK 5 – Publish Findings from Aims 2 & 3 (by end of Year 4)** | | | | |
| 5a. Identify potential publications | *ongoing & planned* | | | |
| 5b. Publish findings from Aim 2 | *ongoing & planned* | | | |
| 5c. Submit findings from Aim 3 | *ongoing & planned* | | | |
| **TASK 6 – Aim 4: Refine & Simplify Model System for Users (Ys 2-4)** | | | | |
| 6a. Initiate cost-analysis of device design | | *ongoing & planned* | | |
| 6b. Seek additional funding for prototyping if needed | | | *planned* | |
| 6c. Initiate analysis of | | *ongoing & planned* | | |

| | | | |
|---|---|---|---|
| proposed scaling algorithms | | | |
| 6d. Integrate injury risk evaluation analysis | | | *planned* |
| 6e. Determine risk evaluation algorithms | | | *planned* |
| 6f. Complete device development | *ongoing & planned* | | |
| 6g. Distribute scaling laws | *planned* | | |
| 6h. Complete software | *planned* | | |
| 6i. Produce exposure guidelines | *planned* | | |

**Task 1**

Work under **Task 1** corresponds to obtaining regulatory approval for both the animal studies (**Task 1a**) and for review of the human data from USAARL (**Task 1b**). Approval has been obtained from both the University of Pennsylvania and USAMRMC for the rat studies. The University of Pennsylvania IACUC approved the animal studies in February 2011 (see Appendix A1 for approval letter from Penn IACUC), and in March 2011, USAMRMC provided their approval based on that IACUC and the ACURO Abbreviated Animal Appendix we submitted. Accordingly, **Task 1a** has been **completed**.

Work under **Task 1b** includes obtaining regulatory approval for use of the human data from USAARL and is ***ongoing***. During the last year, our collaborators at USAARL (Dr. Chancey et al.) have been actively working to obtain such approval but it has been delayed. As recently as September 28, 2011, there was a branch meeting with the Office of Research Protections. During that inspection, USAARL further identified the immediate next steps for obtaining approval for the release of these data. The approval of the use of these data depends on wording of the signed consent forms of the original dataset. As of the final writing of this report date (10/28/11), the original signed consent forms have been located. They were forwarded to the Research Compliance Officer (Jill Emerson) on October 17, 2011 who will be forwarding them to MRMC for review. Accordingly, work related to these data has been limited but is ongoing. We summarize those efforts under Task 2 below.

**Task 2**

Work under **Task 2** corresponds to Aim 1 which broadly is to systematically review epidemiological data of injury exposures and identify those exposures presenting the highest risk to military occupation specialists for low back and neck pain. There were several Tasks associated with review of data which related exposures to symptoms (**Task 2a**) and the review of existing data acquired previously at USAARL (**Task 2b**). Work under **Tasks 2c and 2d** includes running new simulations on the MARS at USAARL, based on the findings from Tasks 2a and 2b. As mentioned above, since approval has been delayed for review of the human data, work on Tasks 2a and 2b are still ongoing and the remaining Tasks in Aim 1 are also delayed. Accordingly, all activities under Task 2 are still ***ongoing*** and ***planned***.

Nonetheless, under Tasks 2a and 2b, during the last year, our collaborators at USAARL have reviewed the contents of the data collected previously by the British Columbia Research Inc. (BCRI). We summarize those data broadly here. All data are de-indentified and are from the BCRI research protocol on repeated mechanical shock. The data are from Phase 2 and Phase 4 of that protocol. Phase 2 involved collecting accelerometer data from ground vehicles in various environments in order to characterize the vibrations and shocks experienced by vehicle occupants. Those data were used to determine a range of shocks (amplitude and frequency) to apply to subjects in Phase 4. During Phase 4, subjects were exposed to a series of mechanical shocks in the x-, y-, and z-axes superimposed on a

background of random vibration. Data from Phase 4 includes experimental and calibration data from one short duration (ST) and two long duration (LT) experiments: **ST1**, **LT3**, and **LT4**. ST1 involved three 35-minute sessions per subject for 10 subjects, LT3 involved a single 420-minute session per subject for 10 subjects, and LT4 involved five 240-minute sessions per subject for 8 subjects. In ST1, the researchers were investigating the relative response to a range of shocks from 0.5 to 4 G and 2 to 20 Hz, in order to determine the frequency and direction of shock most likely to be a health hazard. Also, they were evaluating whether the relationship between shock amplitude and spinal response was linear or nonlinear. The long duration experiments investigated fatigue and recovery in response to repeated shocks for 7 hours in one day (LT3) or 4 hours daily for 5 consecutive days (LT4).

The **experimental conditions** for each case are summarized below:

**ST1** – Individual shocks of amplitudes 0.5 to 4 G and fundamental waveform frequency of 2 to 20 Hz were applied to the subjects in a single axis for each day of testing. Each type of shock was applied twice. A 1.5-minute swept sinusoidal 0.4 G signal from 2 to 40 Hz was applied in each positive axis direction. Shock signals were 5.5-minutes in duration and included 0.5, 1, 2, 3, or 4 G shock magnitudes at 2, 4, 5, 6, 8, 11, 15, or 20 Hz for a single axis and direction.

**LT3** – Motion signatures were 300-seconds long and were repeated for a total time of up to 7 hours. Each 300-second motion signature has 128 2 G shocks (32 x-axis, 32 y-axis, and 64 z-axis) and 2 4 G shocks (z-axis). The fundamental waveform frequency was 6 Hz for all shocks. The 7-hour exposure period was broken down into 4four 105-minute periods, separated by a 15-minute break in the morning, 30-minute break at noon, and 15-minute break in the afternoon.

**LT4** – Motion signatures were identical to LT3 and were repeated for a total exposure time of 4 hours each day for 5 consecutive days. The experiment consisted of two exposure periods of 120-minutes, separated by a 15-minute break each day.

For tests described above, a variety of types of data was acquired using an array of different **instrumentation** approaches. ECG, EMG, force, acceleration, and internal pressure data were collected at 500 Hz. In addition, for the ST1 tests only, positional data were collected using the Optotrak at 200 Hz. Acceleration was measured at the seat and at the thoracic and lumbar spines. The calibration data includes acceleration during a brief pull and release of the skin next to the accelerometer in order to characterize the skin-accelerometer system. EMG electrodes were placed over the muscles of the back at L3 and T9 (right and left erector spinae) and abdominal muscles (right rectus abdominus and right external obliques). Calibration trials included a force transducer attached to a harness at chest level in order to calibrate EMG to force before all experiments and after the LT3 and LT4 tests. Internal pressure was measured during experiments using a rectal pressure probe. Optotrak position data were measured during ST1 using markers on the spinous processes at C7, T4, T6, T8, T9, T10, T12, L1, L5, and on the seat.

In addition, over the past year, we have held monthly conference calls between the two teams at USAARL and Penn in which we review plans for studies, update on activities related to this project at each site, hold journal club reviews, discuss data and provide mutual feedback. We have alternated reviewing manuscripts in the areas of human vibration, transmissibility studies, studies of the physiological response of animals and humans to vibration, and biomechanical studies of dynamics. These conference calls have been very productive in bringing together the multidisciplinary personnel with varied areas of expertise for a common goal of executing this interdisciplinary project. Personnel from Penn who participate in those calls include the PI (Dr. Winkelstein), 2 research engineers, a molecular biologist with expertise in animal behavior, and a post-doctoral fellow with expertise in human spine biomechanics. Personnel from USAARL who participate include Dr. Chancey, a

kinesiologist, an engineer and others as time permits. These routine calls provide the necessary communication to enable integration across the aims of this project.

USAARL is currently preparing a mini-protocol to link the past project under which these data were acquired to our current protocol, so it is ready for IRB review once there is a final determination of the consent forms (see notes above in Task 1b). Therefore, currently Tasks 2a and 2b are still ***ongoing*** and work in the rest of Task 2 remains in the status of ***planned***. However, in the original Statement of Work, Task 2 was planned to occur during Years 1 and 2 of this project so we remain hopeful that we can continue to remain on time for the final deliverables for Aim 1. Moreover, realizing this delay in this Aim, we were able to shift our focus during the last six months to place a greater emphasis on the activities originally planned under Tasks 3 and 4; as such, we are ahead of original projections on the Tasks of those Aims (see below).

**Task 3**

Work under **Task 3** corresponds to Aim 2 of the proposal and focused on developing experimental methods to impose controlled vibration in vivo and to evaluate pain and functional outcomes for vibration and jolt loading to the neck and low back. **Task 3a** focused on the design of an initial device for imposing vibration. The design of such a system required an extensive review of the literature on existing models of vibration in any animal species, human vibration studies, biomechanical and modeling literature, and the effects of mechanical vibration of isolated and whole body on neurophysiology responses. From that review, it was determined that there is very limited available data, and what is available in terms of tolerance to vibration, has largely been performed by exposures of isolated body parts, such as the limb, in rats, or to the rabbit, and turkey [Ariizumi & Okada 1985; Curry et al. 2002; Dina et al. 2010; Maddalozzo et al. 2008; Ogawa et al. 2010]. Only one such study even evaluated pain and it was not related to spinal perturbations [Dina et al. 2010]. Further, from that review a range of exposure parameters were determined for the rat, based on expected physiological outcomes: 0.1 to 0.3 G accelerations, over 0.5 to 3 mm of stroke distance, at frequencies ranging from 1 to 30 Hz. Based on the literature, it was determined that 5 Hz would be a good target frequency, since prior studies with the rabbit and monkey showed resonance to bee at approximately 4.5 Hz [Smith & Kazarian 1994; Weinstein et al. 1987]. The initial device was assembled with a stage mounted on a linear servo-motor (Parker Hannifin, Model MX80L). An accelerometer (Omega Engineering, Model ACC104A miniature quartz accelerometer; 10 mV/G sensitivity) was mounted to the stage. Detailed images of this initial system are provided in Appendix A2.

Based on pilot studies with that initial system, a second system was constructed. The Parker Hannifin MX80L linear servo motor, with a maximum travel length of 25 mm under a 4 N continuous load with maximum of 5 G acceleration was controlled by the Parker Hannifin VIX500IH controller (using Easy-V software). The motor was attached to the vibrating platform (Figure 1) with 2 Dytran (Model 7521A; sensitivity 280 mV/G, weight 3.7 gram) DC accelerometers mounted on the plate at the nose cone and in a strap around the rat's mid-section (Figure 1). An LVDT (MTI Instruments Microtrak II; Model LTC-050-10) with a +/- 5 mm range and sensitivity of 0.8 mm/V is integrated in the system to measure motions of the plate directly. A Phantom VRI-MIROEX1-1024 MM camera (640X480; 500 fps) is integrated with the system to capture imaging of the rat and plate during vibration. All data are synchronized and acquired using National Instruments. Additional images of the system are provided in Appendix A2.

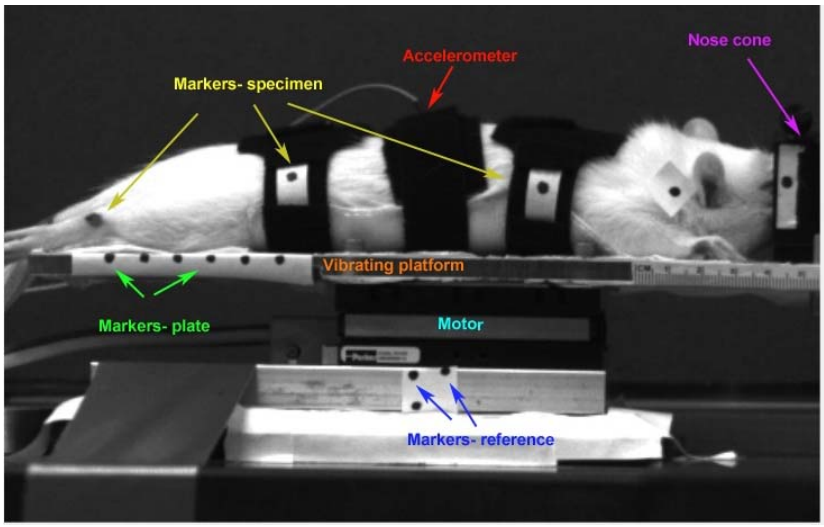


**Figure 1.** Side view of rat in set-up indicating plate, markers, instrumentation, and nose cone for anesthesia.

The mechanical performance of the testing device was evaluated to ensure that it imposed the expected kinematics and kinetics to a rat. The outputs from the accelerometer mounted on the plate and the one placed on the rat's back show very good agreement in both magnitude and phase (Figure 2) regardless of the frequency with which the plate is oscillated. The displacement of the plate is consistent across a range of frequencies and rats (see Appendix A3). Moreover, the variation in displacement is within acceptable ranges from the targeted stroke distance for the plate, remaining below 3%. Using the accelerometer mounted on the plate to estimate displacement, there is very good agreement with both the actual displacement of the plate (as measured by an LVDT) and that measured using the video data, with the primary motion of the rat being in the horizontal direction and the vertical displacement remaining negligible. In particular, the accelerometer mounted on the rat's back is that point with the largest vertical displacement, and even that point's motion up-and-down remains below 0.5 mm (Figure 3). Additional detailed summary data of the horizontal and vertical displacements of rats during a 30 minute exposure of vibration can be found in Appendix A3.

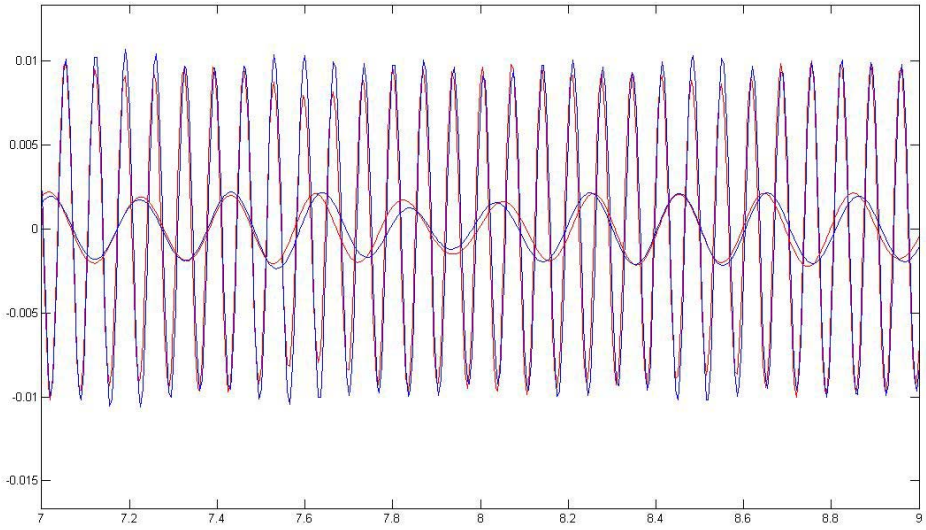


**Figure 2.** Raw signals from the accelerometers mounted on the rat's back (blue) and the plate (red) showing good agreement in magnitude and phase regardless of oscillation frequency.

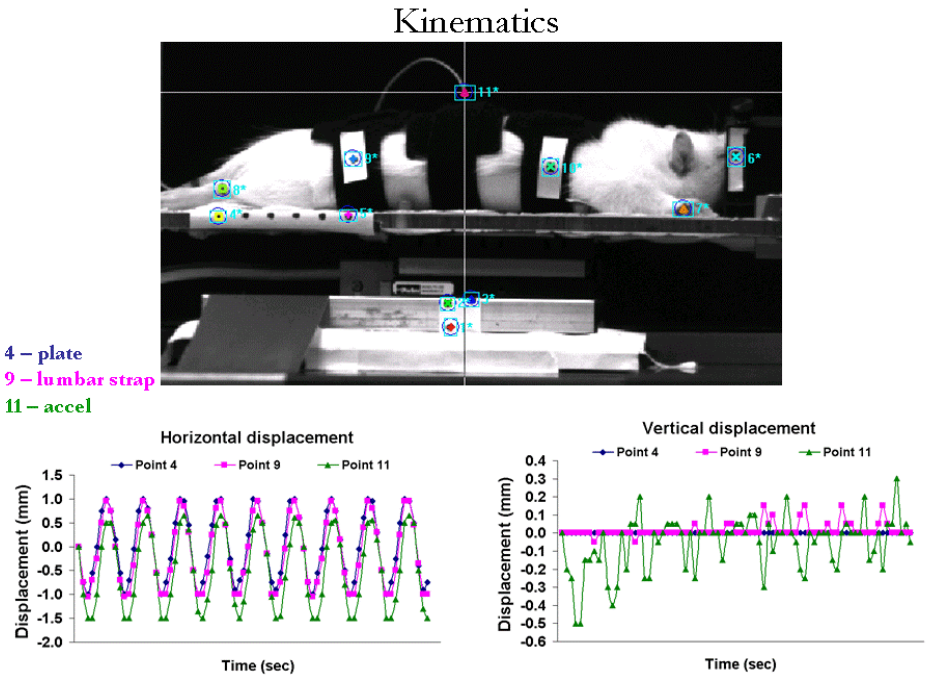


**Figure 3.** Lateral view showing rat and markers used for motion tracking. Below are shown the horizontal and vertical displacements of the plate, lumbar strap and accelerometer.

With these initial biomechanical studies and the first round of redesigns completed (**Task 3a and 3d)** we undertook using this system to carry out pilot in vivo studies (**Task 3c**). The entire system was integrated with inhalation anesthesia to enable sedation of the rat while imposing vibration and imaging of the entire exposure session (Figure 4). A series of pilot studies were performed exposing rats to either 5 or 15 Hz vibration for 30 minutes a day under isoflurane anesthesia. After induction, the rat was attached to the platform using the straps (Figures 1 & 3). The accelerometer was secured to its midsection and its forepaw and ankle were marked for motion tracking (Figures 1 & 3). Video data were acquired for the first minute, for 1 minute starting at 15 minutes, and for the final 1 minute of exposure. This was repeated daily for 7 days. Rats were monitored for weight gain and behavioral assessments daily during the loading exposure period and for 1 week following its termination.

**Figure 4.** Integrated testing system with the camera in the right corner for motion tracking of the platform and rat. Anesthesia is provided through the nosecone on the right which is hooked up to the vaporizer and oxygen on the left of the image.

All rats exhibited steady weight gain during the 14 days, which was comparable to normal increases observed for rats during that time frame. Rats were tested for function using the RotaRod test and exhibited no change in function at any time point, for either the 5 or 15 Hz vibrations. However, both groups of rats exhibited significant ($p<0.05$) increases in sensitivity (ie. pain symptoms) during and following loading in both the forepaw and hind paw (see Appendix A4 for plots). The two vibration frequencies produced similar hyperalgesia symptoms. These studies have been repeated for the 15 Hz exposure and are still being analyzed but indicate the pain symptoms to be a repeatable response. We are currently integrating these findings with pilot studies controlling for daily anesthesia exposure to provide context for the upcoming tissue assays in **Task 4b**. In addition, we are integrating these findings with ongoing mechanical analyses of transmissibility underway in **Task 3b** to identify additional loading exposure frequencies.

**Task 3b** focuses on establishing scaling criteria between the rat and human. Work in the last year has focused in two areas; (1) defining the anatomy and geometry of the rat spine in order to compare the size, shape and relationship of anatomical features to the relevant anatomical features of the human and (2) measuring the resonant frequency of the rat spine for vibration along the long-axis of the spine. Work has been undertaken in both of these areas and is ***ongoing*** as we integrate these findings with the mechanical studies in Task 3g.

In order to define the bony anatomy of the rat, we have been imaging the entire spine using a μCT scanner. Images were taken from the cervical through lumbar region of the spine (n=8 rats), with images taken every 3 μm. Using edge detection methods in Matlab, we are able to parse the relevant bone material from each image (Figure 5). On average, between 2500 and 3400 slices are needed for each rat. Serial images are acquired and radio-opaque beads placed on specified vertebrae help with identifying which level is which for reconstruction and making quantitative measurements (see Appendix A5). Although we are able to make 3D reconstructions of these sections (Figure 6), we are more interested in the quantitative measurements describing the height of the disc, vertebrae, facets, the effective cross-sectional area of the vertebrae and the facet spacing. These imaging studies generate a tremendous amount of data which we are currently still analyzing and comparing to published reports for the human [Stemper et al. 2008].

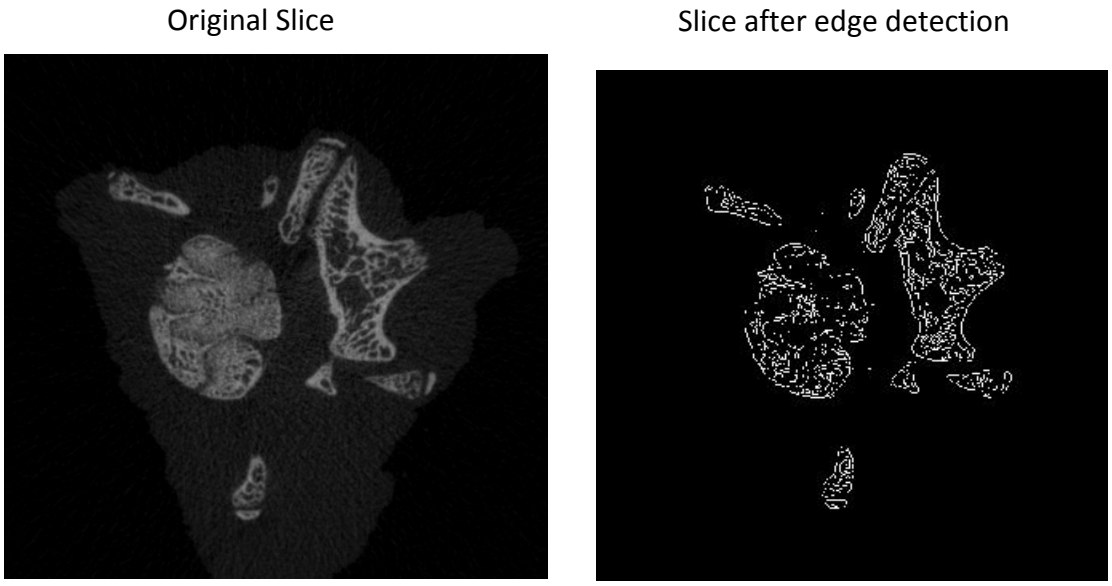


**Figure 5.** Slices from the rat spine showing the original slice and after edge detection.

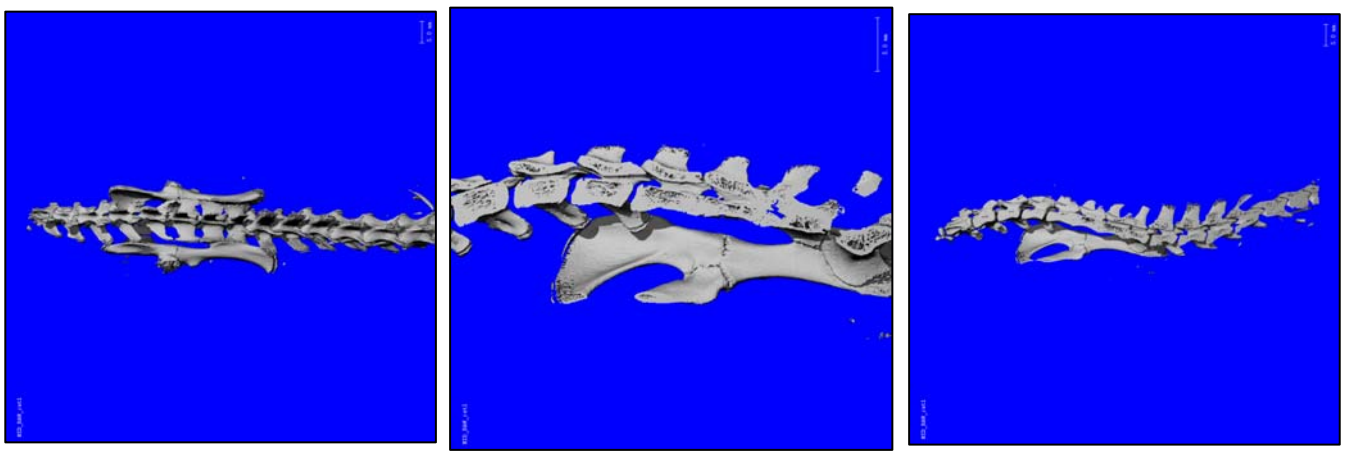

**Figure 6.** 3D reconstructions of the rat spine showing the lumbar spine and pelvis in close up.

As a companion study to the anatomic measurements in **Task 3b**, we have also performed a study of rats to determine the transmissibility response as a function of frequency. These data will provide insight in to the resonance frequency of the rat and also provide comparative data in the context of the human response. Accordingly, 8 expired rats were positioned on our testing platform (Figure 1, 3 & 4) as described above and were exposed to a frequency sweep starting at 3 Hz, going up to 15 Hz in 1 Hz increments. Each vibration was imposed for 1 minute and video, LVDT and accelerometer data were all acquired. A 2-minute rest period was taken between each frequency. The transmissibility was calculated as the RMS acceleration of the rat divided by the RMS acceleration of the platform at each frequency. Interestingly, the data were fairly uniform, with the peak transmissibility at 8 Hz, with 1.4 times the acceleration of the input (Figure 7). Based on our literature review we expected resonance to occur at approximately 5 Hz. Accordingly, moving forward with additional studies in Task 3e, we are looking to pilot in vivo studies with the vibration frequency at 8 Hz, based on these data. Further, the fact that the transmissibility is below 1 for both 5 Hz and 15 Hz, and both of those exposures induced sustained pain responses in vivo, suggests that the energy must be absorbed by tissues in those cases. This may provide a potential mechanism for the generation of pain. Coupling these findings with ongoing tissue assays in Task 4 will begin to provide such further insight.

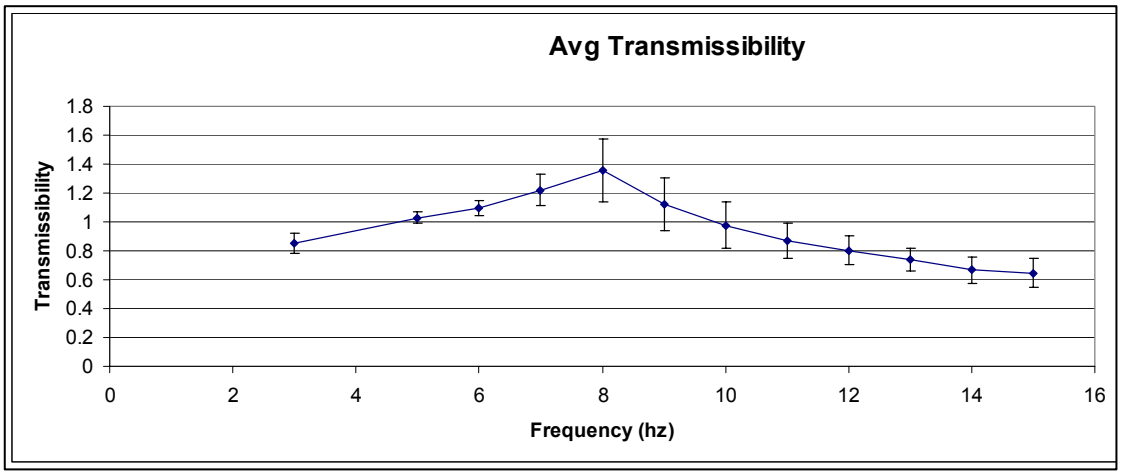


**Figure 7.** Transmissibility of rats exposed to vibration along the long axis of the spine.

Together, both of these studies in the rat provide data for making direct comparisons to human data. Using those similarities and differences we will continue to undertake relevant scaling, together with our modeling efforts in Task 3g to develop scaling algorithms for both the biomechanical insults to

the spine and the relevant thresholds for modifications in the physiological function. While the basic data acquisition for the scaling studies is completed, we continue with these studies in order address work under Task 6c (see below).

**Task 3e** is ongoing now that we have such encouraging data for the continuous exposure of 5 and 15 Hz. Studies are planned to investigate the effect of a single jolt of comparable severity and of exposure to jolt following initial sensitization by daily exposure. These studies are ***ongoing and planned*** in order to determine the full set of loading conditions for the in vivo studies to be completed later in **Task 3f**.

In addition to the analysis of the kinematics and kinetics already described above for the vibration studies in vivo and the transmissibility studies under **Task 3b**, we have begun developing lumped mass models simulating our vibration system, with a set of governing equations to describe a 1- or more- degree of freedom system. Please see Appendix A6 for the models and equations. Using the 1-degree of freedom model and assumptions of the mass based on the average weight of the rat, we are able to begin to make estimates of predicting displacements for different forcing frequencies and use that model to simulate and predict the resonant frequency (Figure 8). While the accuracy of any such model depends on the assumptions and constants used for the stiffness and dissipative elements in the rat, work under Task 3g is ongoing and will be coupled with studies in Task 3b to provide a more realistic and useful model to analyze and extrapolate the mechanical exposures to those not induced in the rat studies and to be integrated for use in predicting the human response. Activities under **Task 3g** are ***ongoing*** and will be completed according to the original time line by the end of Year 3.

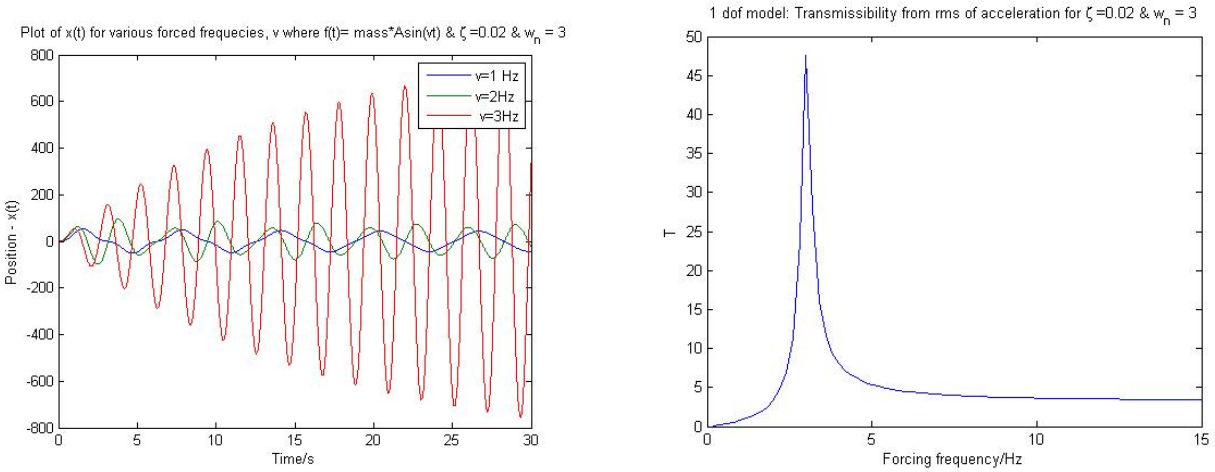


**Figure 8.** Outputs from a lumped mass model simulating the vibration applied to the rat. The plot on the left shows the change in displacement with change in forcing frequency and the plot on the right shows the transmissibility as a function of frequency, with the predicted resonance frequency at approximately 3 Hz.

The majority of our activities during the past year under Task 3 have focused on developing the testing set-up, behavioral assessments and identifying exposure protocols that may and may not induce pain. We have made great progress on those fronts and identified several additional studies that provide added value to either biomechanics, injury or pain research. While we have not yet presented publications on this work, we are now poised to report on several of these studies and are currently preparing 3 abstracts on each of the model development, scaling data and transmissibility study presented in Tasks 3b and 3c above. We will submit abstracts to the Northeast Bioengineering Conference in January 2012 and are preparing 2 additional full length manuscripts that we expect to submit in the next 6 months.

**Task 4**

Work under **Task 4** corresponds to Aim 3 which involves the temporal characterization of responses in relevant tissues of the spine following the vibration exposure. The majority of these activities are scheduled for Years 2 through 4 owing to the need to establish the injury conditions first under Task 3. However, given that we have already identified two exposure conditions that induce sustained pain, we have initiated tissue assays of a variety of tissues from those pilot studies to help identify which tissues and which biochemical mediators may be most relevant for studies under **Task 4**. We summarize those initial efforts here as early efforts on **Task 4b**. However, we note that **Tasks 4a and 4c-4e** are currently in the planning stages.

From studies in Task 3 we have harvested a variety of tissues, including the brain, cervical and lumbar spinal cord enlargement, cervical and lumbar discs, paraspinal muscles in both regions of the spine and the gastrocnemius muscle since it is close to the region where behavioral sensitivity was measured in Task 3. Further, when available, we also harvested DRG samples but due to their small size it is not always possible. A complete itemization of tissues harvested to date is provided in Appendix A7. We have not yet probed the brain tissue. We have probed the spinal cord for astrocytic activation using GFAP, and inflammation using COX2, both by western blot. Data are still being quantitatively analyzed for statistical comparisons. However, in the cervical disc samples it appears that pro-NGF is significantly ($p=0.01$) increased following vibration compared to controls, but not different between vibration groups. Moreover, PAR2 (a receptor for thrombin that has been implicated recently in pain) exhibits a similar response with a significant increase compared to control following vibration ($p=0.018$) and a further significant difference between the two vibration frequencies with 15 Hz inducing a nearly two-fold significant (p-0.04) increase over levels following a 5 Hz vibration. Interesting, the lumbar discs did not exhibit a similar difference in PAR2 expression, suggesting there may be different loading and/or different thresholds for regulating modifications in this response between the two regions of the spine.

Expression of a variety of biochemicals appears to modulated by vibration in the gastrocnemius muscle. For example, COX2 expression is reduced by nearly 15% for both vibration frequencies compared to control, and this is significant ($p=0.048$). In contrast, only the 5 Hz exposure increases the pro-inflammatory cytokine IL1 in that same muscle, which was elevated above both control and 15 Hz exposure values ($p=0.02$). Different isoforms of nerve growth factor appear to exhibit different responses, which is requiring additional assays and has led us to also harvest muscle tissue for immunohistochemistry assays moving forward with studies in this Aim. Certainly, these early findings are still limited in terms of sample numbers, but they provide direction for our ongoing and future assays under this Aim and also provide support that tissue responses may be modulated in association with the onset and maintenance of pain induced by this type of vibration exposure.

Some studies in **Task 4** have been initiated already to provide tissue at day 7 following the termination of loading (n=7 or 4 per group). Additional studies are ***ongoing*** and are ***planned*** for the remainder of the project period. However, based on the data already from the 15 Hz exposures in Task 3, we are beginning to prepare two manuscripts, one that compares the effects of vibration frequency on spinal inflammation and the other which evaluates NGF and PAR in the discs. We expect to submit these two papers in the next year and that several more will follow with the continued studies in Tasks 3 and 4.

**Task 5**

Work under **Task 5** corresponds to identification of publications for work from Aims 2 and 3 and is ongoing. It will be completed by the end of Years 3 and 4 as detailed in the original statement of work. Please also see the Reportable Outcomes section for additional details.

**Task 6**

Work under **Task 6** corresponds to Aim 4 which broadly consists of efforts to make the model system and software available as resources for the broader scientific community. The majority of the specific sub-tasks of that Aim are largely planned for Years 2-4 of this project. However, given our early successes in developing a working system and identifying the conditions for use in Aim 3, we have also initiated **Task 6a** and **Task 6c**. In particular, with a working test system in place for imposing vibration injury, we have already initiated cost-analysis of device design. Current costs associated with the vibration system are estimated to be approximately $15,000 (See Appendix A8 for detailed itemization). We continue these analyses and are investigating more economic options for components of our device and will continue these ***ongoing*** efforts for **Task 6a** over the next year. Some studies in **Task 6c** have been initiated already, using data from our μCT studies in Aim 2. With the anatomic datasets acquired under **Task 3b** and the mathematical models we are developing in **Task 3g**, we are performing quantitative analysis and comparisons of rat and human kinematic and kinetic responses to begin analysis and development of scaling algorithms for Task 6c. These efforts are ongoing and will continue as originally projected to be completed before or by the end of Year 3.

All **other sub-tasks of Task 6** are planned for completion during or by the end of Year 4, according to the original timeline.

## KEY RESEARCH ACCOMPLISHMENTS

- Established experimental test set-up for imposing controlled vibration to the live rat
- Developed several vibration exposure protocols that induce behavioral sensitivity (ie. pain) that is sustained following the termination of exposure.
- Determined the resonant frequency of the rat spine to be 8 Hz for vibration along the long-axis of the spine.
- Collected imaging data of the rat spinal anatomy for quantitative description of the size and shape of its individual tissue components.
- Developed an initial lumped parameter mathematical model of the spine.
- Determined that the pain response does not differ between 5 Hz and 15 Hz vibration exposures but some aspects of the tissue responses do exhibit differences.
- Established methodology to enable assay of spinal disc materials.
- Collected a wide-array of spinal tissues for assaying of the inflammatory, nociceptive and injury responses.

## REPORTABLE OUTCOMES

***Manuscripts, Abstracts & Presentations***

1. Guarino BB, Baig HA, Branconi JA, Jaumard NV, Winkelstein BA. Repeated daily exposure to whole body vibration induces sustained widespread behavioral sensitivity. *Northeast Bioengineering Conference*, to be submitted January 2012.
2. Gohkale A, Guarino BB, Baig HA, Winkelstein BA. The rat spine as an anatomical surrogate for the human: A μCT study. *Northeast Bioengineering Conference*, to be submitted January 2012.
3. Baig HA, Guarino BB, Winkelstein BA. A transmissibility study of the rat spine. *Northeast Bioengineering Conference*, to be submitted January 2012.

4. Guarino BB, Baig HA, Branconi JA, Jaumard NV, Winkelstein BA. Repeated daily exposure to whole body vibration induces sustained widespread behavioral sensitivity and spinal inflamamtion. *To be submitted.*
5. Baig HA, Guarino BB, Winkelstein BA. A transmissibility study of the rat spine: A potential relationship to pain.. *To be submitted*.
6. Branconi JA, Guarino BB, Baig HA, Winkelstein BA. Vibration along the spine induces permanent modifications of nerve growth factor and PAR2 in the disc that are associated with persistent pain. *To be submitted*.

***Animal Model Generated***

1. Protocol developed for inducing sustained sensitivity following repeated daily vibration to the rat.

## CONCLUSION

There is currently very little definitive mechanistic data defining the relationship between whole body or spine vibration, physiological mechanisms and pain. Considering that pain is tremendous problem especially for those who sit for extended periods of time while traveling over rough terrain wearing heavy helmets and body armor, a novel model platform for studying how such exposures produce chronic pain can provide tremendous utility for providing insight about exposures with risk for producing pain. We **hypothesized** that a model of vibration and/or jolt induced pain could be produced in the rat that would simulate the human exposures. **Studies already completed under this award support our original hypothesis and have importance in moving the entire project forward**. Among the major findings of importance include the fact that even 30 minutes of vibration a day for only 7 days is sufficient to induce significant widespread behavioral sensitivity that is sustained for at least a week following the termination of vibration. A second major important finding is that a host of biochemical changes appear to be present in association with pain and some may be sensitive to the frequency of vibrations. The modifications in the cervical disc appear to be the most significant so far but only pilot studies have been performed so far. Interestingly, while the resonant frequency of the rat was expected to be around 5 Hz based on literature reviews, our own analysis of transmissibility indicates the resonant frequency to be around 8 Hz, which suggests that the response to that vibration may be even more pronounced. ***Together and individually, all of these findings are quite novel and have tremendous implications for both sub-failure spinal injury and pain.*** In addition, they establish a strong and exciting foundation for the remaining in vivo and human studies which expand these studies to include additional exposures and to define the time course of physiological responses in the whole animal system.

Based on the activities during the last year, we do not have any modifications to the future work, only to recommend slight changes to the timing of activities for the future work. As indicated above, activities to obtain regulatory approval for the review of human data is still ongoing. This has also delayed our progress to date on Aim 1 (Task 2). Therefore, in the past year we shifted efforts to more aggressively work on Task 3 so that Task 2 could be spread out over Years 2 and 3 given this unforeseen delay in reviewing the USAARL data. We believe once that approval is obtained, work on those studies will move rapidly and will be facilitated by the strength of our already heavily integrated collaborative teams. We continue our monthly conference calls to continue to discuss efforts in those studies and to prepare for the work should approval be granted. We continue to move all efforts forward as best as possible and will compensate for this delay by expended extra effort in other areas of this project.

Current risk assessment algorithms for pain and injury rely largely on speculative notions and standards for injuries that may not be relevant. Although vibration is a common experience while riding

in vehicles, and standards have been developed to protect Soldiers from repeated jolts, they are not sufficient for current designs, nor do they address neck injury potential or the mechanisms by which tissue loading produces pain and/or injury. Also, there is no clear understanding of the physiological consequence of repeated sub-threshold loading to lowering the pain threshold. Accordingly**,** it is necessary to develop an in vivo model that mimics the biomechanical loading to the body in order to study how loading produces tissue injury, which tissues are injured, how pain develops, and which conditions place the military specialists at greatest risk for injury. The new knowledge gained from such a novel injury/pain model has direct utility for evaluating injury risks and developing potential therapeutics. Our findings to date already provide evidence that even low level vibration is sufficient to produce pain and sustained modifications in the spinal cord and throughout the musculoskeletal tissues of the body. Our in vivo and mathematical models that have already been developed under this project have tremendous promise for providing major benefit to the military by identifying tissues at risk for injury and exposures which pose the greatest threats to producing pain.

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## APPENDIX

**A1.** IACUC Approval letter from Penn.

Penn
University of Pennsylvania

**Office of Regulatory Affairs**
IACUC Protocol Administration

**Troy M. Hallman, MS, VMD, Diplomate ACLAM**
Director of Animal Welfare, Office of Regulatory Affairs

3624 Market Street, Science Center ♦ Suite 301S ♦ Philadelphia, PA 19104 ♦ Phone: 215-573-2540 ♦ Fax: 215-573-9438

**INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)**
(Multiple Project Assurance # A3079-01)

BETH A WINKELSTEIN
1301 - Bioengineering
210 S. 33rd Street
Room 240 Skirkanich Hall
PHILA, PA 19104

04-Feb-2011

| | |
|---|---|
| PRINCIPAL INVESTIGATOR | : BETH A WINKELSTEIN |
| PROTOCOL TITLE | : Development of a Novel Translational Model of Vibration Injury to the Spine to Study Acute Injury in Vivo |
| GRANT TITLE | : Development of a Novel Translational Model of Vibration Injury to the Spine to Study Acute Injury in Vivo |
| SPONSORING AGENCY | : DEPARTMENT OF THE ARMY |
| PROTOCOL # | : 803407 |

Dear DR. WINKELSTEIN:

With receipt of the requested revisions for the above protocol your study now stands fully approved as of **14-Jan-2011**. Work may begin at any time. This study will be due for review on or before **14-Jan-2014**. Protocols are only valid for three years from the date of approval. Please use **Ben Reports (https://galaxy.isc-seo.upenn.edu/ws/benreports)** on a routine basis to check the status of your protocols.

**If notification of IACUC review is required by the funding source required, please notify our office in writing of the contact person, agency name, address, phone number, fax number, and email as soon as possible.**

**Please take note of the following information:**

**Personnel Training:** It is the responsibility of the Principal Investigator to ensure that all persons have completed all necessary IACUC and EHRS training prior to participating in the research described in this protocol.

**Amendments*:** If you wish to change any aspect of this study, such as procedures, sponsor, analgesics, anesthetics, or the investigators, please communicate your requested changes in writing to the Director for Regulatory Affairs. The new procedures cannot be initiated until Committee approval has been given.

**Reapproval*:** It is the investigator's responsibility to apply for reapproval of ongoing research annually for protocols involving USDA covered species, or more often if required by the funding agency.

*Forms for amendments and re-approval (Form B) are available from the Office of Regulatory Affairs web site [http://www.upenn.edu/regulatoryaffairs].

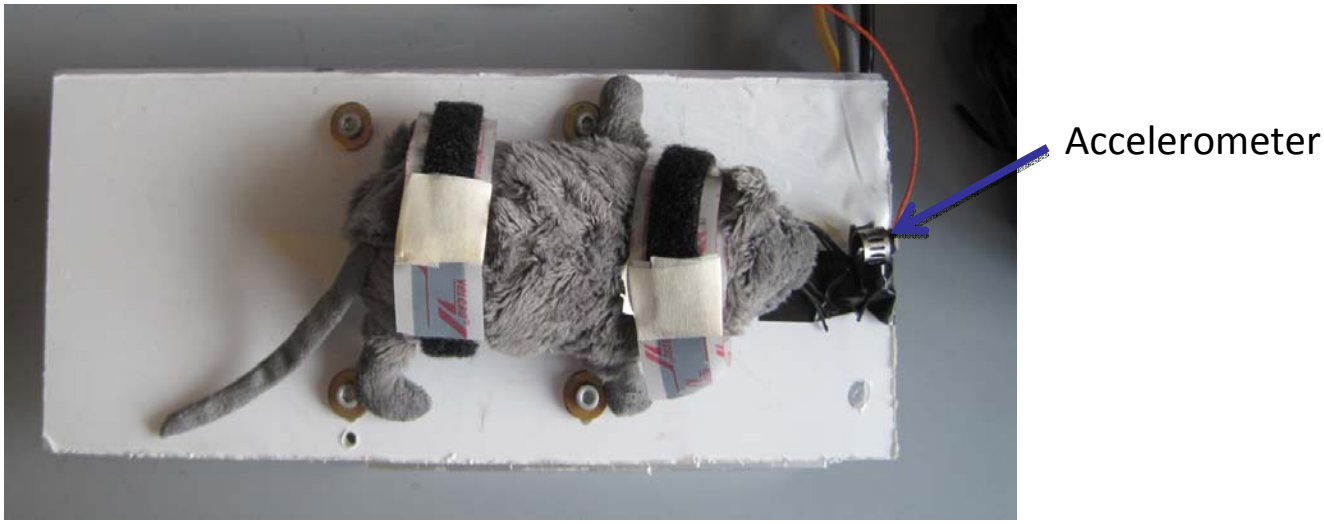
**Completion of Study:** Please notify the Director for Regulatory Affairs as soon as the research has been completed.

Thank you for your cooperation with the Committee.

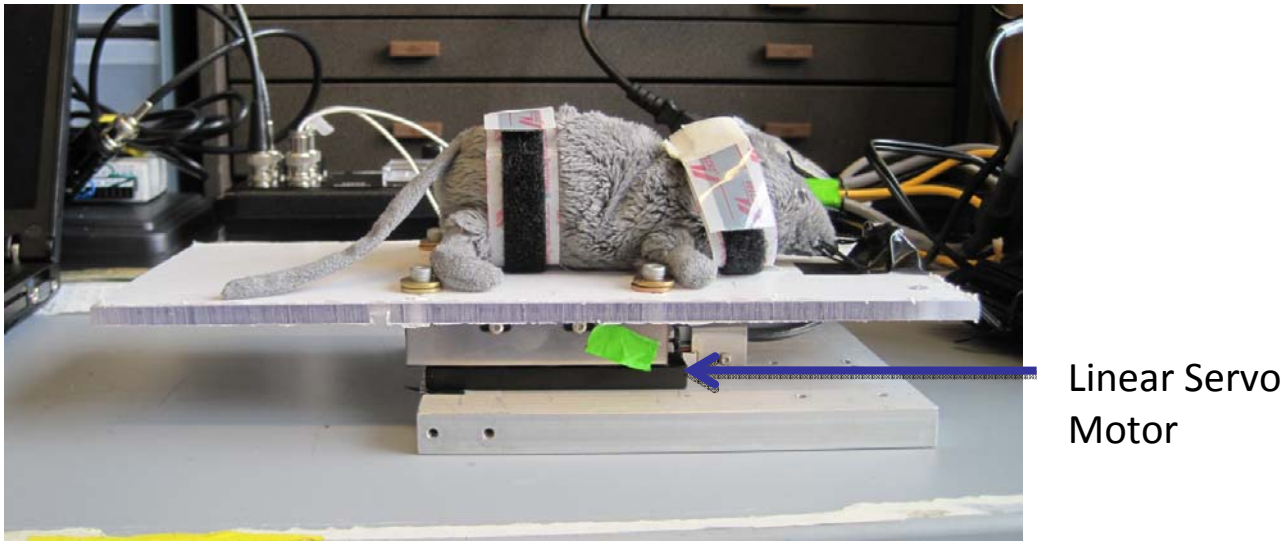
Sincerely,

Troy Hallman, MS, VMD
Director of Animal Welfare, IACUC

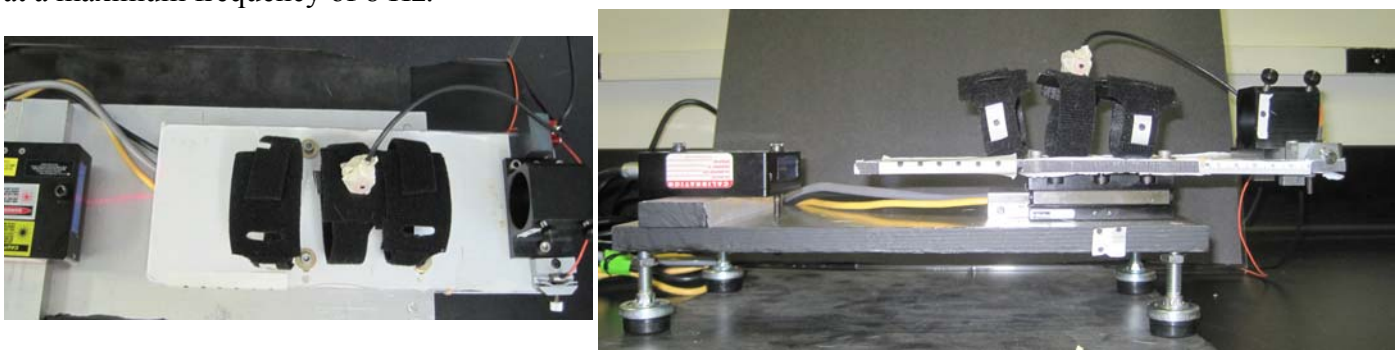
**A2.** Images of Initial Device (Task 3a)




Top view of initial vibration device with rat surrogate shown and straps to ensure coupling to the stage. The accelerometer was mounted on the stage, as indicated.

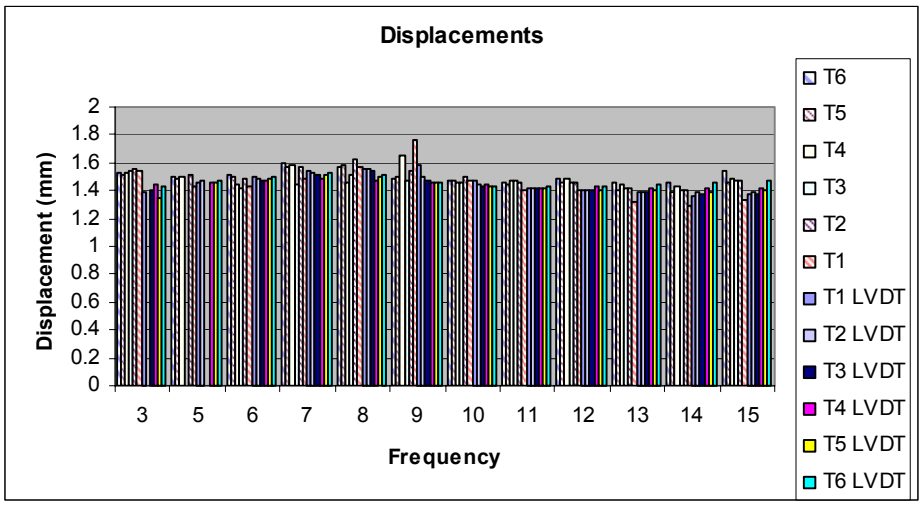



Side view of initial vibration device with rat surrogate shown. Under the stage is the linear servo motor which enabled movement in the left-right direction of the image, with a peak-to-peak amplitude of 3 mm at a maximum frequency of 8 Hz.

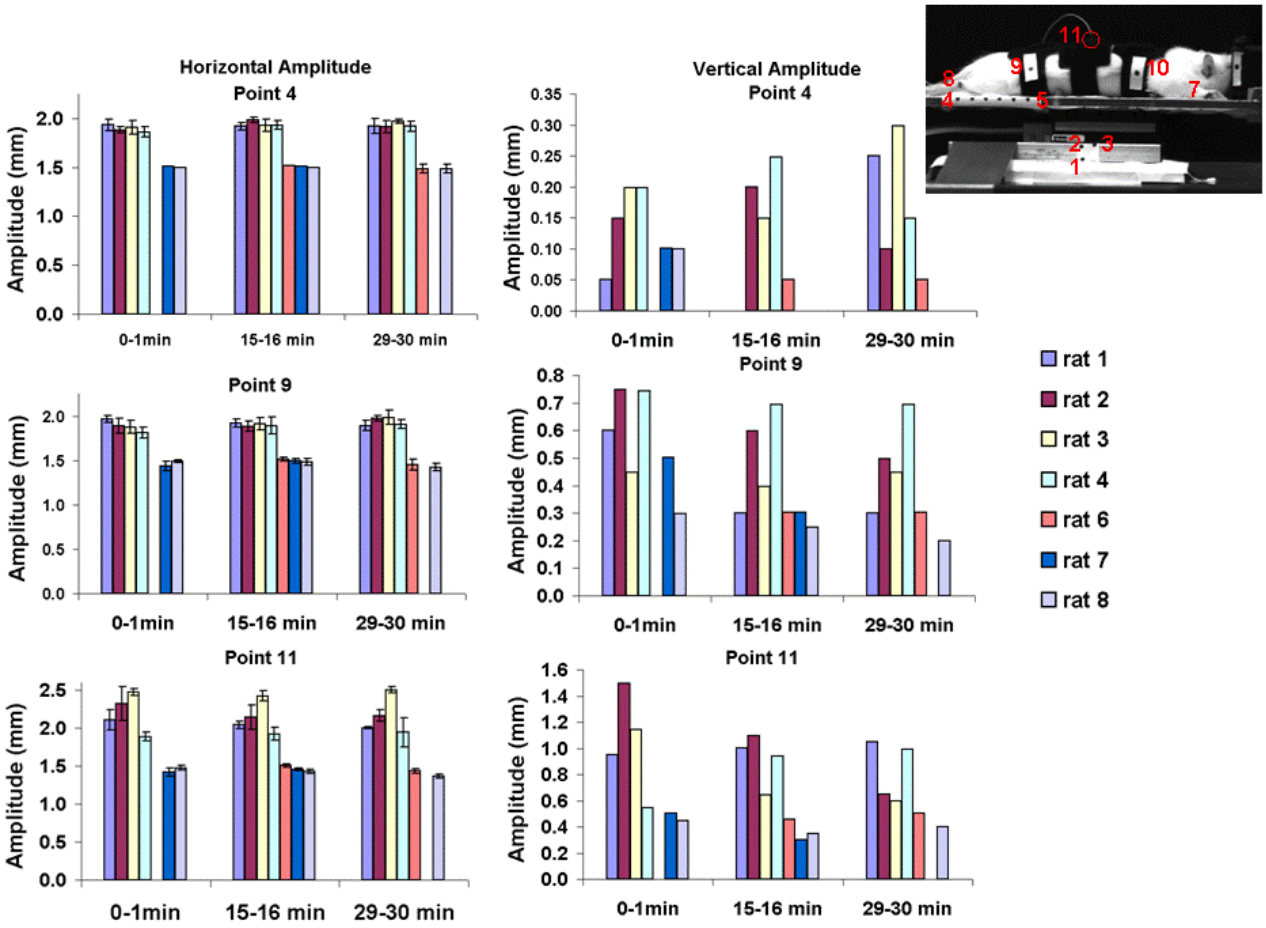


Top (left) and side (right) views of modified device showing laser to measure displacement, stage with markers, straps to hold the rat, accelerometer mount on spine, nosecone for inhalation anesthesia, stage accelerometer.

**A3.** Summary of Displacements (Task 3a)

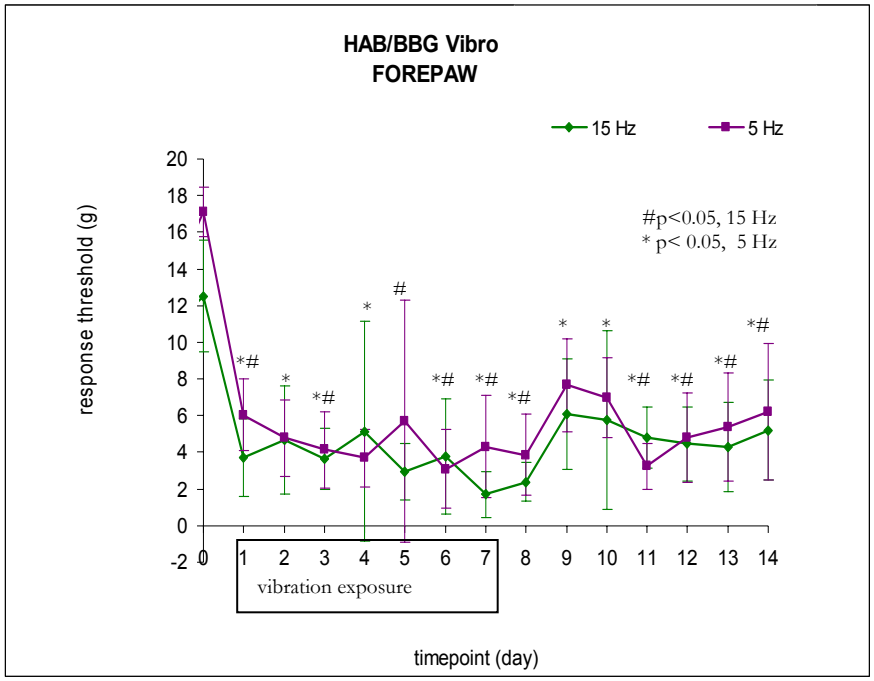



Comparison of plate displacements using accelerometer and LVDT data showing good agreement across a range of frequencies from 3 to 15 Hz.

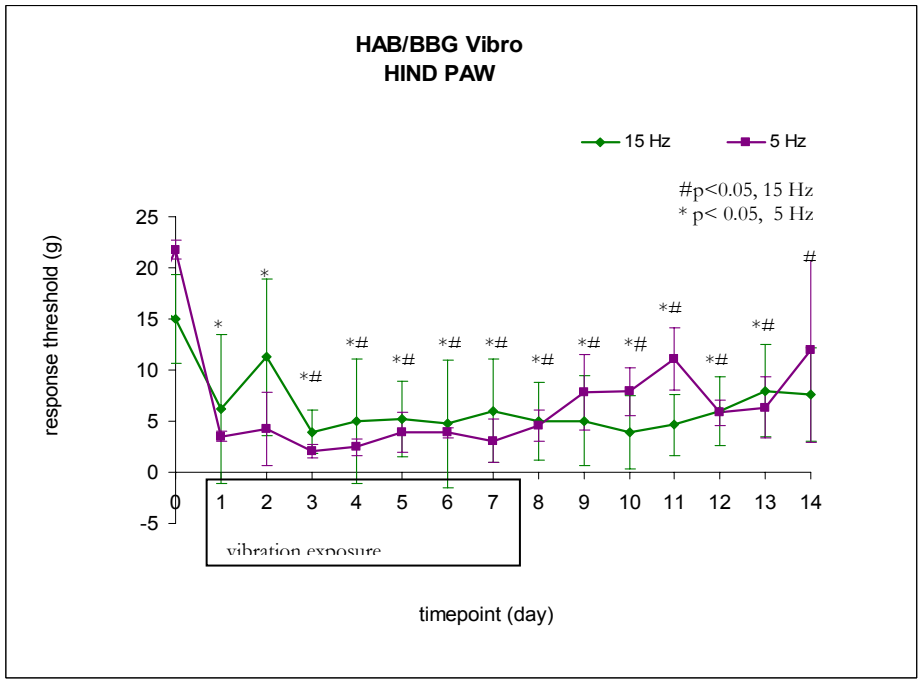



Horizontal and vertical displacement of rats determined using image data during a 30 minute vibration exposure, showing consistency across the beginning, middle, end of the 30 minutes and being lower for points tracked on the plate and strap, with the accelerometer (point 11) exhibiting the greatest vertical fluctuation. Rats here were exposed to either 5 or 15 Hz of vibration.

**A4.** Mechanical Hyperalgesia for Pilot Studies (Task 3c)

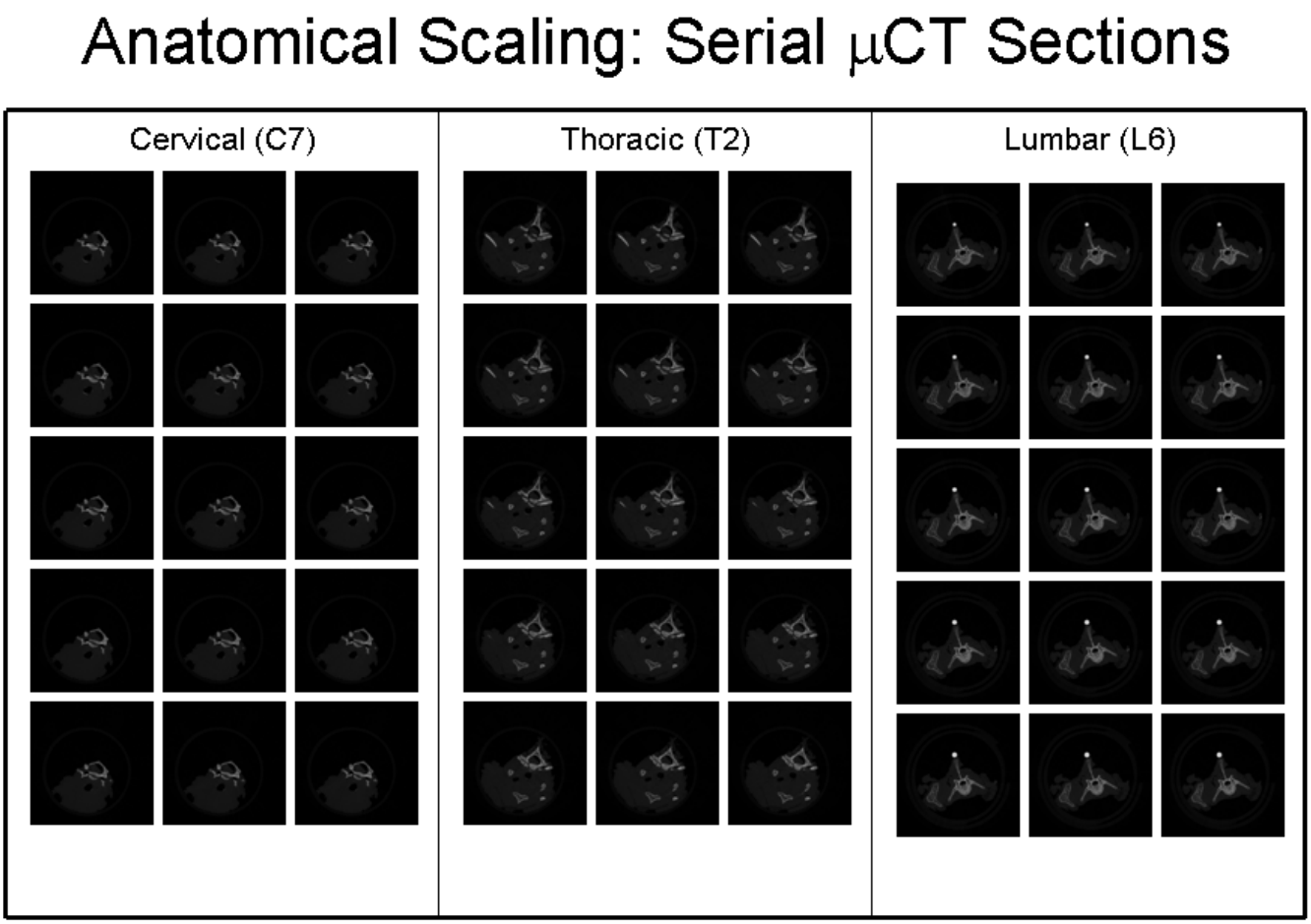



Sensitivity in the forepaw is induced immediately following a single exposure to vibration, which remains even following termination of vibration.



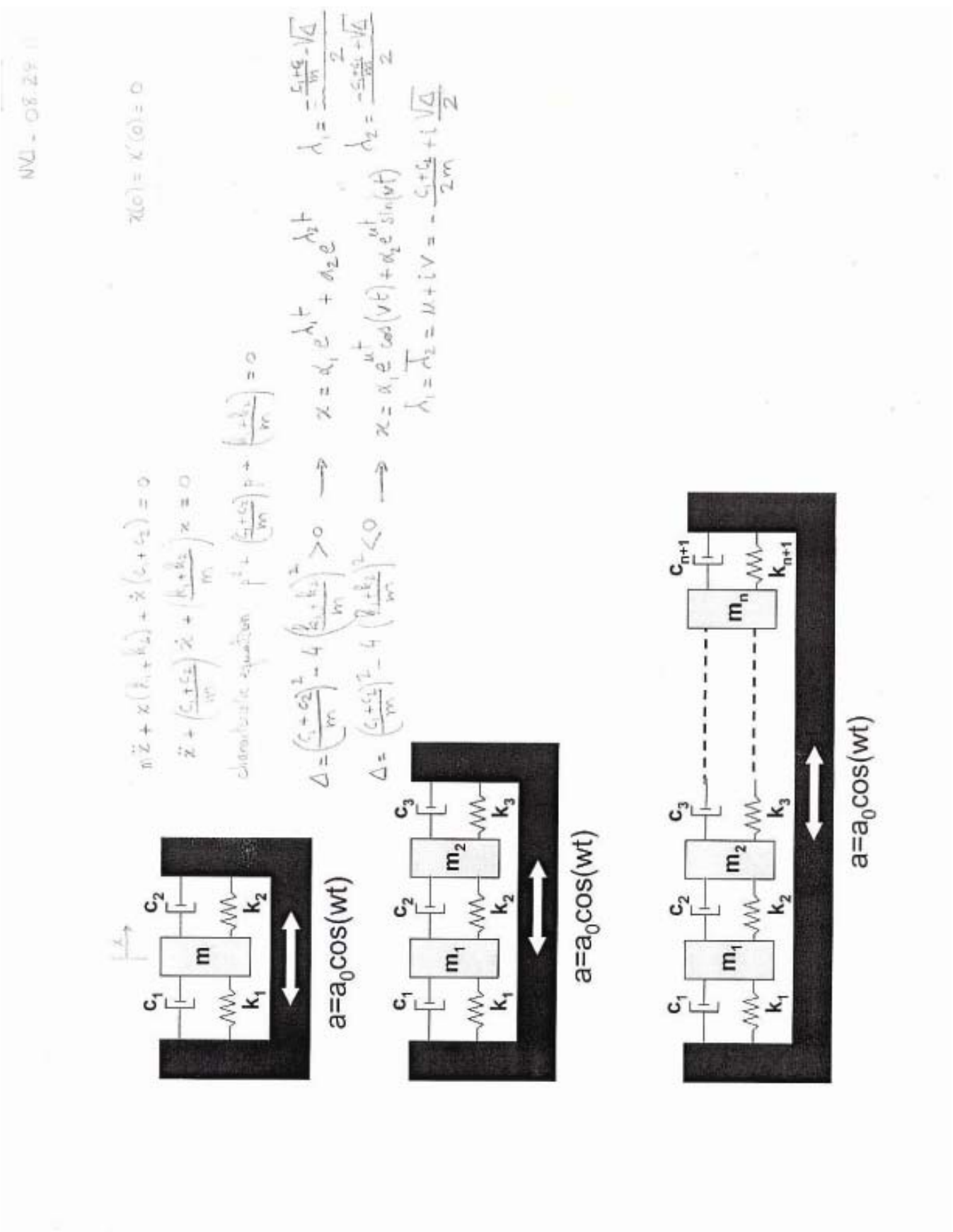

Sensitivity in the hindpaw is induced immediately following a single exposure to vibration, which remains even following termination of vibration.

**A5.** Serial Imaging of Rat Spine (Task 3b)




Serial images of the rat spine in the cervical, thoracic and lumbar region. Images are taken every 3 μm. Markers are placed on known spinal levels to ensure registration with anatomic features. The white ball in the Lumbar images is such a marker on the spinous process.

**A6.** Lumped Parameter Models of Vibration (Task 3g)



For $\Delta > 0$

$$x = \alpha_1 e^{\lambda_1 t} + \alpha_2 e^{\lambda_2 t}$$
$$\dot{x} = \alpha_1 \lambda_1 e^{\lambda_1 t} + \alpha_2 \lambda_2 e^{\lambda_2 t}$$
$$\ddot{x} = \alpha_1 \lambda_1^2 e^{\lambda_1 t} + \alpha_2 \lambda_2^2 e^{\lambda_2 t}$$

For $\Delta < 0$ most likely the case

$$x = \alpha_1 e^{ut}\cos(vt) + \alpha_2 e^{ut}\sin(vt)$$
$$\dot{x} = \alpha_1 u e^{ut}\cos(vt) - \alpha_1 v e^{ut}\sin(vt) + \alpha_2 u e^{ut}\sin(vt) + \alpha_2 v e^{ut}\cos(vt)$$
$$= (\alpha_1 u + \alpha_2 v) e^{ut}\cos(vt) + (\alpha_2 u - \alpha_1 v) e^{ut}\sin(vt)$$
$$\ddot{x} = (\alpha_1 u + \alpha_2 v) u e^{ut}\cos(vt) - (\alpha_1 u + \alpha_2 v) v e^{ut}\sin(vt) + (\alpha_2 u - \alpha_1 v) u e^{ut}\sin(vt) + (\alpha_2 u - \alpha_1 v) v e^{ut}\cos(vt)$$
$$= \left[(\alpha_1 u + \alpha_2 v) u + (\alpha_2 u - \alpha_1 v) v\right] e^{ut}\cos(vt) + \left[(\alpha_2 u - \alpha_1 v) u - (\alpha_1 u + \alpha_2 v) v\right] e^{ut}\sin(vt)$$
$$= \left[\alpha_1 u^2 + \alpha_2 vu + \alpha_2 uv - \alpha_1 v^2\right] e^{ut}\cos(vt) + \left[\alpha_2 u^2 - 2\alpha_1 uv - \alpha_2 v^2\right] e^{ut}\sin(vt)$$

$$\ddot{x}(0) = \alpha_1 u^2 + 2\alpha_2 vu - \alpha_1 v^2 = 0$$
$$x(0) = \alpha_1 = 0$$

$$\ddot{x}(t) = -\alpha_2 u^2 e^{ut}\sin(vt)$$

**A7.** Detailed Inventory of Tissues Harvested to Date (Task 4b)

# Tissue Inventory

| Rat | Brain | Gastrocnemius | Cervical Enlargement | Lumbar Enlargement | Cervical Disc | Lumbar Disc | Lower Paraspinal Muscle | Upper Paraspinal Muscle | C-DRG | L-NR DRG |
|---|---|---|---|---|---|---|---|---|---|---|
| 15 Hz | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 4 | 2 | 1 |
| 5 Hz | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 1 |
| Anes Control | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | | |
| Normal | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 1 |

**A8.** Detailed Itemization of Costs of Device (Task 6a)

| | |
|---|---|
| Motor | $1600 |
| Controller | $1000 |
| Accelerometers | $ 866 |
| Power Supply | $ 350 |
| National Instruments | $ 250 |
| Camera | $9900 |
| Lens | $ 895 |
| **TOTAL** | **$14,861** |